Monitoring *Xylella fastidiosa* for changes in host range and virulence using genome-based methods.

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Combating the threat of the plant pathogenic bacterium *Xylella fastidiosa* using genome-based methods linked to national and international monitoring.

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Relevance to PD?

- Obtaining baseline genetic data on which forms of *Xylella* damage which plant hosts.
- Establishing a monitoring system to identify changes in geographic and/or host range of *Xylella*.
- Identify the genetic changes that lead to shifts in virulence and/or plant host.

Initial Questions

- How great are the genetic differences between the various types of *Xylella*?
- How host-specific are they?
- How do new plant-host variants arise?
- How much variation exists for virulence within types?

Host range of *Xylella fastidiosa* in N. America

Host range is very extensive – just consider some of the "named" diseases:

Pierce's	Almond leaf scorch
Phony peach	Alfalfa dwarf
Plum leaf scald	Elm leaf scorch
Mulberry leaf scorch	Oak leaf scorch
Periwinkle wilt	Ragweed stunt
Red maple scorch	Sycamore leaf scorch
Oleander leaf scorch	Blueberry leaf scorch

What is the genetic basis of this host range?

Several years ago, it was clear that there were distinct "pathovars" BUT they did not necessarily form into useful genetic groupings:

PD forms can cause ALS

Some ALS forms do not cause PD

OLS forms do not cause PD or ALS

CVC & CLS forms can cause PD

Almeida & Purcell (2003); Purcell et al (1999); Li et al (2002).

Genetic Differences

- Schaad et al (2004) suggested 3 subspecies, based primarily on DNA-DNA hybridization:

subsp. fastidiosa - N. American PD
(isolates from grape, alfalfa, almond, maple)

subsp. multiplex - N. American ALS
(isolates from almond, peach,elm,plum, sycamore)

subsp. pauca - S. American CVC

- Schuenzel et al (2004) suggested recognizing one more:

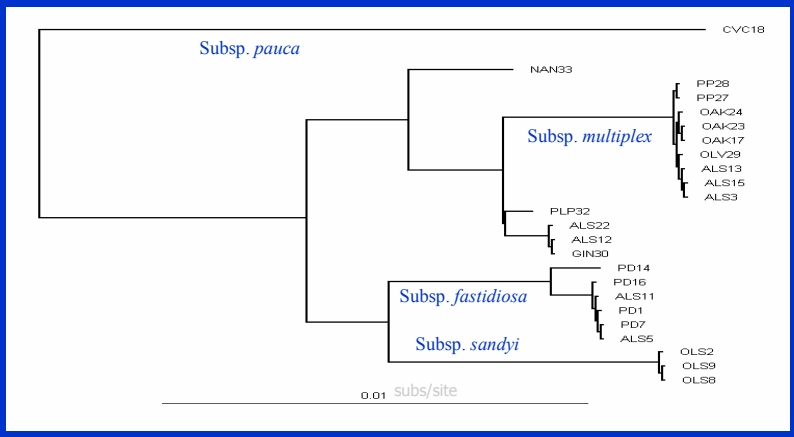
subsp. sandyi - N. American OLS
(from oleander, plus day lily, magnolia, jacaranda)

How "real" are these differences?

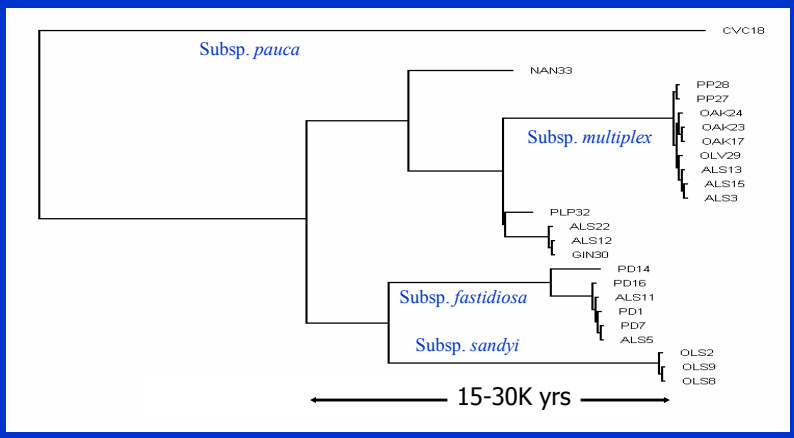
	fastidiosa	sandyi	multiplex	pauca
fastidiosa	**85%**	Not tested	**58%**	**41%**
sandyi	**2.59%** **0.42%**	Not tested	Not tested	Not tested
multiplex	**3.25%** **0.70%**	**3.18%** **0.81%**	**84%**	**45%**
pauca	**6.85%** **1.22%**	**6.85%** **1.22%**	**6.85%** **1.22%**	**87%**

Top: DNA-DNA hybridization (Schaad et al 2004)
Bottom: synonymous (upper), non-synonymous (lower)
(Schuenzel et al 2005)

DNA sequence data – based on 9 genes



What is the time scale?



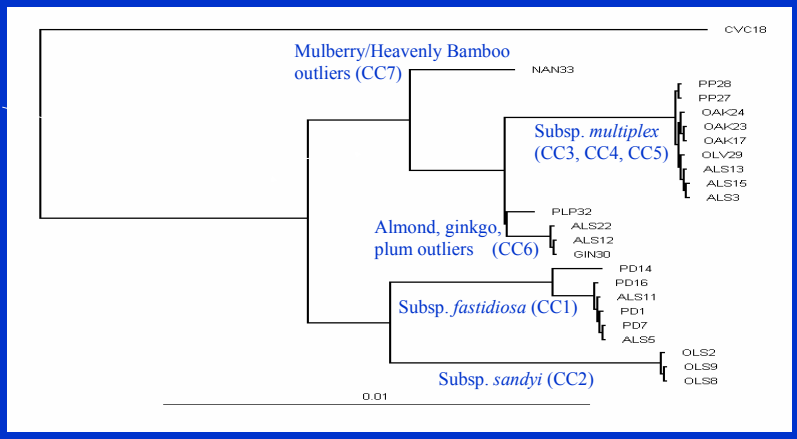
see Schuenzel et al (2005)

Multi-Locus Sequence Typing (MLST) Maiden et al (1998)

- Based on DNA sequences of around 500bp from 7 predefined housekeeping genes. The variants (alleles) of each gene are numbered 1 onwards.
- Each isolate is classified as a "**sequence type**" by the 7 allele numbers, e.g. 2, 1, 4, 1, 2, 7, 1 (2nd allele at gene 1, 1st allele at gene 2, etc).
- Results made available on central internet database - assists in communication
- Provides resolution usually appropriate for identifying important genetic groupings called "**clonal complexes**".

Alternate genetic approaches, e.g. SSR – may be very important for more finely subdividing groups.

MLST subdivision in Clonal Complexes



Scally et al (2005)

Do "outliers" undermine subspecies?

1. The subspecific designations seem justified based on genetic divergence and age.
2. Are the outliers additional old taxonomic groups?

   No – their origins appear to be relatively recent. They are inter-subspecific recombinants.

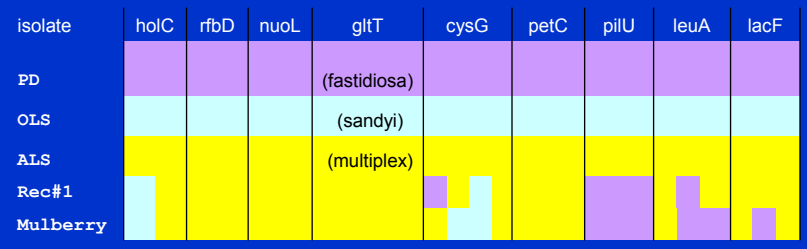
Inter-Subspecific Recombination

Two types of multiple recombinants have shown up several times in independent isolates.

The recombinants carry material from all three N. American subspecies, and (more surprisingly) the specific recombination events are often identical.

Rec #1: 2xalmond, ginkgo, crape myrtle, and plum (1 each)

Mulberry: 4 isolates, also 1 from *Nandina*.



How does recombination occur?

1. Precise homologous recombination (no additional nucleotides at the ends).
2. How long are the recombined regions? Based on an on-going whole-genome analysis – assuming a minimum detectable length of 300bp - the mean size is about 450bp, with max of about 1500bp.

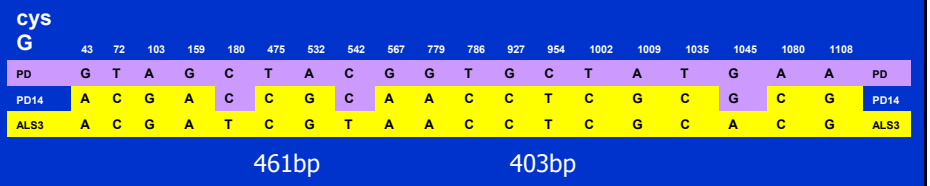
Suggests transformation – Is the DNA picked up in the sharpshooter or in the plant host?

Why care about recombination?

- Recombination can result in very rapid evolution – such as dramatic changes in virulence or host range.
- It is possible that the invasion of GWSS into California may promote a higher level of recombination, by repeatedly bringing the different subspecies into close proximity.

Recombination in PD strains

Although the two multiple inter-subspecific recombinants are derived from subsp. *multiplex* and have never been found in grape, subsp. *fastidiosa* is not immune from recombination:



We need to assess the virulence of these novel genotypes.

Assessing Virulence and Host Specificity

We have begun to develop a standard protocol to assess the host range and virulence of specific sequence types.

Our goal is to simultaneously test interesting/novel isolates plus at least one control isolate against a panel of host plants.

Where possible, this will be done both in California and in Florida.

To establish some baseline results we are testing:

Temecula, Dixon, Ann1, and a Mulberry isolate against merlot, pinot, almond, oleander, mulberry, oak, elm, & crapemyrtle.

Where do we go from here?

1. We would REALLY like researchers to either type their isolates under the MLST scheme or send us DNA and we will gladly do it.
2. Develop a "fool-proof" way of obtaining *Xylella* DNA from infected plants – "fool proof" meaning that even I could do it.
3. Encourage growers/advisors to send us samples from any unusual outbreaks of scorch-like disease.
4. Make all the information we can gather on the distribution of the various types of *Xylella* available on the web (**www.xylella.org**)